Released by/Date Effective:

Author Name	Title	Signature/Date

Approver Name	Title	Signature/Date

1. PURPOSE

1.1. The purpose of this procedure is to describe the proper use, maintenance and handling of the NanoDrop 1000 Spectrophotometer

2. SCOPE

2.1. This procedure applies to the HPV Serology Laboratory located at the Advanced Technology Research Facility, C2007.

3. REFERENCES

3.1. NanoDrop 1000 Spectrophotometer user manual

3.2. HSL_EQ_021.01: NanoDrop 1000 Use and Maintenance Form

3.3. HSL_EQ_021.02: NanoDrop 1000 Calibration Form

3.4. HSL_EQ_019: Use and Maintenance of the Milli-Q Integral 3 Water System

3.5. HSL_GL_001: Waste Disposal at the Advanced Technology Research Facility

3.6. HSL_GL_002: Equipment Qualification and Calibration in the HPV Serology Laboratory

3.7. HSL_GL_003: Good Documentation Practices for the HPV Serology Laboratory

3.8. HSL_GL_004: Laboratory Notebook Control and Use for the HPV Serology Laboratory

3.9. HSL_GL_006: Reagent Preparation for the HPV Serology Laboratory

3.10. HSL_GL_007: Reagent and Chemical Expiry in the HPV Serology Laboratory

3.11. HSL_GL_008: Laboratory Flow and Gowning Procedures for the HPV Serology Laboratory

3.12. HSL_GL_009: HPV Serology Laboratory BSL-2 Procedures

3.13. HSL_GL_010: Control and Request of Documents in the HPV Serology Laboratory

4. RESPONSIBILITIES

4.1. The Research Associate, hereafter referred to as analyst, is responsible for reviewing and following this procedure.

4.2. The Scientific Manager or designee is responsible for training personnel in this procedure and reviewing associated documentation.

4.3. The Quality Assurance Specialist is responsible for quality oversight and approval of this procedure.

5. REAGENTS, CHEMICALS AND EQUIPMENT

5.1. NanoDrop 1000 Spectrophotometer

5.2. CF-1 Calibration Fluid (ThermoFisher Scientific, Cat # CHEM-CF-1 or equivalent)

5.3. PR-1 Pedestal Reconditioning Compound (ThermoFisher Scientific, Cat # CHEM-PR1-KIT or equivalent)

5.4. Type I Water

5.5. Kimwipes (VWR, Cat # 21905-026 or equivalent)

6. HEALTH AND SAFETY CONSIDERATIONS

6.1. Proper safety precautions should be taken while working in a laboratory setting. This includes, but is not limited to, proper protective equipment such as lab coats, safety glasses, closed-toe shoes, and non-latex gloves.

6.2. Refer to the respective SDS when working with any chemicals.

6.3. Refer to “HSL_GL_001: Waste Disposal at the Advanced Technology Research Facility” regarding waste disposal processes at the ATRF.

7. DEFINITIONS

Term	Definition
FME	Facilities, Maintenance and Engineering
HPV	Human Papillomavirus
HSL	HPV Serology Laboratory
SDS	Safety Data Sheets
SOP	Standard Operating Procedure
Type I Water	Ultrapure/Reagent Grade/Critical applications

8. OPERATION

8.1. The following volumes are sufficient to ensure reproducibility:

8.1.1. Aqueous solutions of nucleic acids: 1.0 µL

8.1.2. Purified protein: 2 µL

8.1.3. Bradford, BCA or Lowry assay: 2 µL

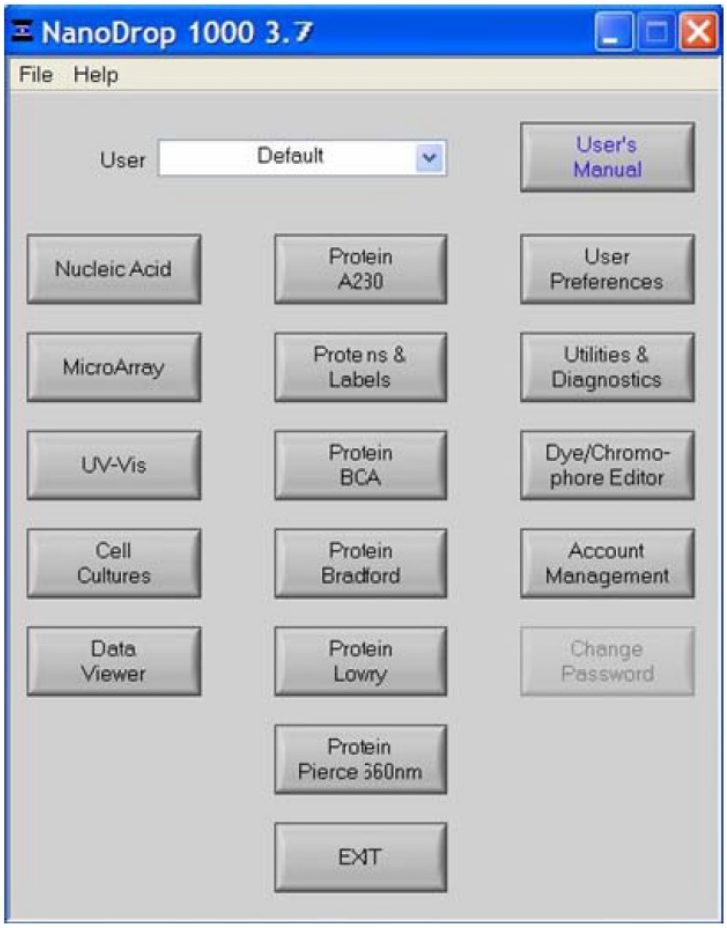
8.1.4. Microbial cell suspensions: 1-2 µL

8.2. Open program ND-1000 Version 3.8. A window message will pop-up asking to initialize the instrument. Click **OK**. With the sample arm open, wipe both the upper and lower pedestals with kimwipe, damp with nuclease free water (Type I water).

8.3. Load 2 µL of Type I water sample onto the lower measurement pedestal and close the sampling arm then click **OK**.

8.4. The message "**Initializing Spectrometer- please wait**" will appear. When this message disappears, the instrument will be ready for use.

8.5. A window menu with the following option is available:



8.5.1. Nucleic Acid – concentration and purity of nucleic acid

8.5.2. MicroArray – dye incorporation concentration and purity of nucleic acid

8.5.3. UV-Vis – general UV-Vis measurements

8.5.4. Cell Cultures – “absorbance” (light scattering) measurement of suspended microbial cells

8.5.5. Protein A280 – concentration and purity of purified protein

8.5.6. Proteins & Labels – concentration of dye-labeled proteins, conjugates, and metalloproteins

8.5.7. Protein BCA – protein concentration using the BCA assay

8.5.8. Protein Bradford – protein concentration using the Bradford assay

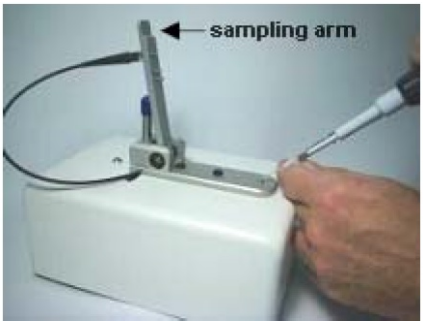
8.5.9. Protein Lowry – protein concentration using the Modified Lowry assay

8.5.10. Pierce 660 nm Protein Assay – protein concentration using the new 660 nm assay

8.6. Click on which sample type is being measured from the main menu.

8.7. With the sample arm open, wipe both the upper and lower pedestals with kimwipe, damp with Type I water.

8.8. With the sample arm open, pipette the sample onto the lower measurement pedestal. (see picture below)



8.9. Close the sampling arm and initiate a spectral measurement by clicking the **Measure** button of pressing the **F1** button.

8.10. When measurement is complete, open sampling arm and wipe the sample from both the upper and lower pedestals with kimwipe.

8.11. Wipe both the upper and lower pedestals with kimwipe, damp with Type I water.

8.12. Sample data from all application modules are automatically stored in archive files and can be opened by either the integrated Data Viewer software program or spreadsheet programs such as MS Excel.

9. MAINTENANCE

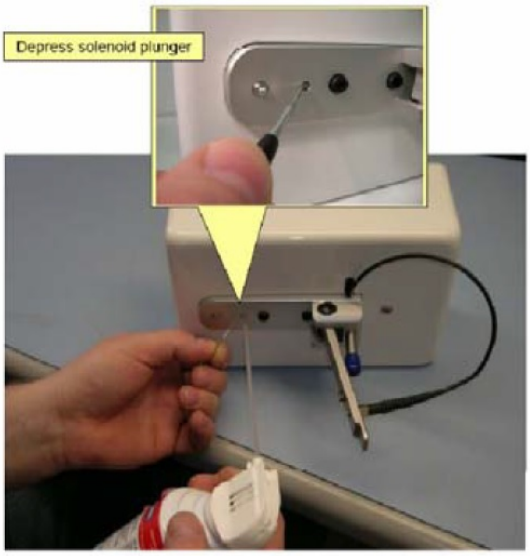
9.1. Yearly Maintenance:

9.1.1. Removal of lint build-up under the solenoid:

9.1.1.1. Lay the instrument on its side with the source fiber (black fiber optic cable facing up) (see Picture below).

9.1.1.2. Open the arm of the sampling mechanism.

9.1.1.3. Using a paperclip or a small screwdriver, manually depress the solenoid plunger and spray compressed air down the solenoid plunger hole. Be sure to keep the can of compressed air upright so as not to spray the propellant into the instrument.



9.1.1.4. After cleaning, lay the instrument back upright.

9.1.1.5. Document maintenance performed on HSL_EQ_021.01: NanoDrop 1000 Use and Maintenance Form.

9.1.2. Reconditioning the pedestals:

9.1.2.1. Open the vial containing PR-1 and use the applicator provided in the kit to remove a pin-head sized amount of the compound.

9.1.2.2. Apply a very thin, even layer of PR-1 to the surface of the upper and lower pedestals.

9.1.2.3. Wait 30 seconds for the PR-1 to dry.

9.1.2.4. Fold a clean, dry laboratory kimwipe into quarters and remove the PR-1 by aggressively rubbing the surface of the upper and lower pedestals until all compound residue is removed.

Note: The black appearance of the removed residue is normal.

9.1.2.5. The reconditioning process is complete once the laboratory wipe is clean of black residue.

9.1.2.6. To check the effectiveness of the reconditioning, load 1 µL of Type I water onto the lower measurement pedestals and visually verify that the water sample beads on the pedestal.

9.1.2.7. Document maintenance performed on HSL_EQ_021.01: NanoDrop 1000 Use and Maintenance Form.

9.2. Bi-Annual Calibration Check:

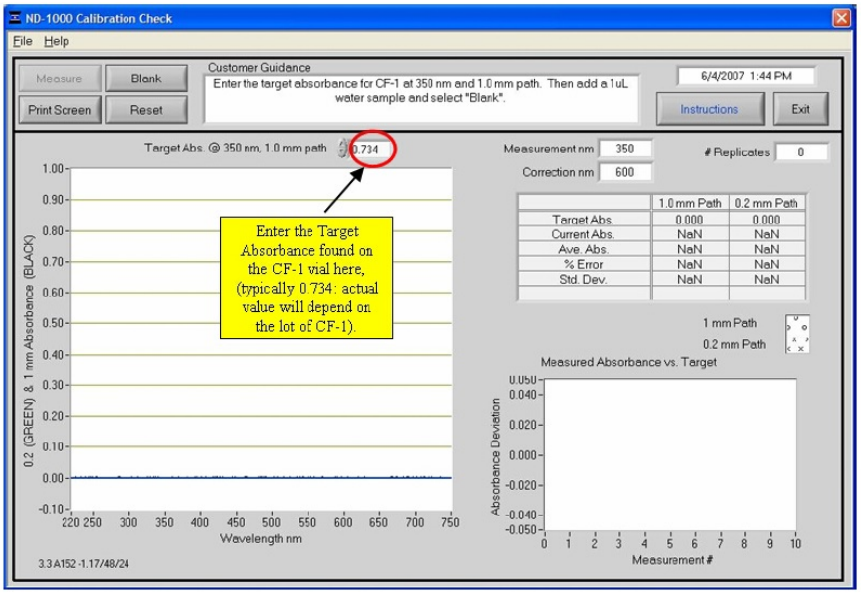
9.2.1. Perform calibration check every six months with CF-1 fluid.

9.2.1.1. Wipe both the upper and lower pedestals with kimwipe, damp with Type I water.

9.2.1.2. After ensuring the measurement pedestals are clean and that a 1 µL water sample beads on the lower pedestal, open the NanoDrop 1000 Spectrophotometer Calibration Check Software and follow the prompts in the Customer Guidance text box of the software.

9.2.1.3. Enter the Target Absorbance found on the CF-1 vial as directed in the image below.

Note: The target absorbance will depend on the lot of CF-1 in use, so care should be taken to enter the Target Absorbance located on the individual vial of CF-1.



9.2.1.4. Wipe both the upper and lower pedestals with kimwipe.

9.2.1.5. Add 1 µL of Type I water and select "**Blank**".

9.2.1.6. After the blank measurement is done, wipe both the upper and lower pedestals with kimwipe.

9.2.1.7. Before opening the ampoule of CF-1 Calibration Fluid, shake vigorously to ensure solution is thoroughly mixed. Ensure all solution is collected in the bottom portion of the ampoule.

9.2.1.8. Carefully break the neck of the ampoule to open the CF-1 Calibration Fluid.

9.2.1.9. Follow the on-screen prompts in the Customer Guidance text box. Using individual 1 µL aliquots of the CF-1 Calibration Fluid, measure 10 replicates. After the 10th measurement, the calibration check results will be displayed on-screen in the Customer Guidance text box.

Note: The CF-1 Calibration Fluid is supplied in a single use vial. The CF-1 must be used within one hour of opening the vial. Exposure to the environment or transferring of the fluid to another container may cause a significant change in concentration.

9.2.1.10. If the instrument does not pass the calibration check using a 1 µL sample size, immediately repeat the procedure (Step 9.1.3.9) using 2 µL aliquots. If calibration check still fails using 2 µL aliquots, contact Scientific Manager as instrument vendor may need to be contacted.

9.2.1.11. To print a copy of the results, click the "**Print Screen**" button. A .JPG of the final results is automatically archived. Store the printout in the associate equipment Raw Data Binder, and label with the following information:

Logbook Reference
Pass/Fail
Analyst Date/Initials
Reviewer Date/Initials

9.2.2. Document maintenance performed on HSL_EQ_021.02: NanoDrop 1000 Calibration Form.

10. ATTACHMENTS

10.1. Not applicable.

11. REVISION HISTORY

Revision Start Date	Version #	Changes	Reasons
05Apr17	New	Create new SOP for use and maintenance of NanoDrop 1000 spectrophotometer	Currently no SOP

Frederick National Laboratory for Cancer Research sponsored by the National Cancer Institute	HPV Serology Laboratory Standard Operating Procedure	
NanoDrop 1000 Spectrophotometer Use and Maintenance Form		
Form ID: HSL_EQ_ 021.01 Associated SOP: HSL_EQ_021	Version 1.0	Page 1 of 1

Equipment ID:	Calibration Date:	Calibration Due Date:

Date	Initials	Reagent Used/ Lot Number	Activity Performed/ Comments
		□ N/A, Reagent: _____ □ Lot #: _____	
		□ N/A, Reagent: _____ □ Lot #: _____	
		□ N/A, Reagent: _____ □ Lot #: _____	
		□ N/A, Reagent: _____ □ Lot #: _____	
		□ N/A, Reagent: _____ □ Lot #: _____	
		□ N/A, Reagent: _____ □ Lot #: _____	
		□ N/A, Reagent: _____ □ Lot #: _____	
		□ N/A, Reagent: _____ □ Lot #: _____	

Review By/Date:	
QA Review By/ Date:	

Frederick National Laboratory for Cancer Research sponsored by the National Cancer Institute	HPV Serology Laboratory Standard Operating Procedure	
NanoDrop 1000 Calibration Form		
Form ID: HSL_EQ_ 021.02 Associated SOP: HSL_EQ_021	Version 1.0	Page 1 of 1

Equipment ID:	Calibration Date:	Calibration Due Date:

Reagent	Lot Number	Expiration Date
CF-1		
Time Vial Opened	Open:	Used:

	Equipment ID	Calibration Due Date
Pipette: μL		

Amount of Calibration Sample:

☐ 1.0 μL ☐ 2.0 μL

Time of Last Reading: _____

☐ PASS

☐ FAIL

Comments: ☐N/A

Analyst/Date:	
Review By/ Date:	